Minireview

Actin's propensity for dynamic filament patterning

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Abstract Actin, through its various forms of assembly, provides the basic framework for cell motility, cell shape and intracellular organization in all eukaryotic cells. Many other cellular processes, for example endocytosis and cytokinesis, are also associated with dynamic changes of the actin cytoskeleton. Important prerequisites for actin's functional diversity are its intrinsic ability to rapidly assemble and disassemble filaments and its spatially and temporally well-controlled supramolecular organization. A large number of proteins that interact with actin, collectively referred to as actin-binding proteins (ABPs), carefully orchestrate different scenarios. Since its isolation in 1994 [Machesky, L.M. et al. (1994) J. Cell Biol. 127, 107–115], the Arp2/3 complex containing the actin-related proteins Arp2 and Arp3 has evolved to be one of the main players in the assembly and maintenance of many actin-based structures in the cell (for review see [Borths, E.L. and Welch, M.D. (2002) Structure 10, 131–135; May, R.C. (2001) Cell Mol. Life Sci. 58, 1607–1626; Pollard, T.D. et al. (2000) Rev. Biophys. Biomol. Struct. 29, 545–576; Welch, M.D. (1999) Trends Cell Biol. 11, 423–427]). In particular, when it comes to the assembly of the intricate branched actin network at the leading edge of lamellipodia, the Arp2/3 complex seems to have received all the attention in recent years. In parallel, but not so much in the spotlight, several reports showed that actin on its own can assume different conformations [Bubb, M.R. et al. (2002) J. Biol. Chem. 277, 20999–21006; Schoenenberger, C.-A. et al. (1999) Microsc. Res. Tech. 47, 38–50; Steinmetz, M.O. et al. (1998) J. Mol. Biol. 278, 793–811; Steinmetz, M.O. et al. (1997) J. Cell Biol. 138, 559–574; Millonig, R., Salvo, H. and Aebi, U. (1988) J. Cell Biol. 106, 785–796] through which it drives its supramolecular patterning, and which ultimately generate its functional diversity.

Key words: Actin; Branching; Bundling; Dimer; Nucleus

1. Probing actin polymerization by chemical cross-linking

The polymerization of globular monomeric actin (G-actin) into F-actin filaments has been extensively studied in vitro using a variety of techniques. In principle, a nucleation-condensation step at the onset of the reaction precedes an elongation phase, during which actin subunits associate and dissociate with distinct kinetics at the two ends of the growing filament until a steady state is reached.

In earlier studies, our laboratory has analyzed the formation of small oligomers in the course of actin polymerization by chemical cross-linking using the bifunctional sulfhydryl reagent *N*,*N*′-1,4-phenylenebismaleimide (1,4-PBM) [9,10], which has been shown to cross-link adjacent subunits in prepolymerized actin filaments [11,12]. Examples of a time course of actin polymerization monitored by 1,4-PBM cross-linking are illustrated in Fig. 1. Although the kinetics vary depending on the salt conditions chosen, the first intermolecular cross-linked species that is detected after the initiation of polymerization always migrates with an apparent molecular mass of ~86 kDa on SDS–PAGE. With ongoing polymerization, this 86 kDa band gradually diminishes and instead a second cross-linked species with an apparent mass of ~120 kDa appears. Based on their respective electrophoretic mobilities the initial dimer was called 'lower dimer' (LD) and its successor 'upper dimer' (UD). The appearance of LD is transient, which might explain why it has so far eluded detection in vivo. Sedimentation equilibrium centrifugation of the two purified cross-linked oligomers corroborated that they are both dimers [10].

The time required for the shift from LD to UD is critically dependent on the polymerization conditions. If 100 mM KCl is used to induce polymerization of pure Ca-G-actin (Ca^{2+} bound to the high-affinity divalent cation binding site, *HAS*) at a concentration of 1 mg/ml, the shift from LD to UD production begins around 5–10 min after adding the salt (Fig. 1A). With ongoing polymerization the UD is the only dimer made, together with larger oligomers. If polymerization is induced by 50 mM $MgCl_2$, the shift from LD to UD takes place as early as 30 s after salt addition (Fig. 1B). It is noteworthy that electron microscopy revealed a tendency of actin filaments to laterally associate into paracrystalline arrays under these salt conditions [10,13]. When Mg^{2+} is bound to the HAS (Fig. 1C), UD formation is rapid, too. These cross-linking experiments illustrate that the assembly of LD as the first dimeric species and the subsequent shift to UD production is an intrinsic feature of actin polymerization and not an effect of the divalent cation occupying the HAS or the polymerization conditions chosen. Further evidence that the assembly of LD is an intrinsic property of all actins is provided by cross-linking studies with actin isolated from *Dictyostelium discoideum* [14]. Despite differences in the polymerization kinetics between rabbit skeletal muscle and cytoplasmic actin from *Dictyostelium* [14], LD remains the fastest produced dimer.

In a recent paper, Bubb and colleagues reported that

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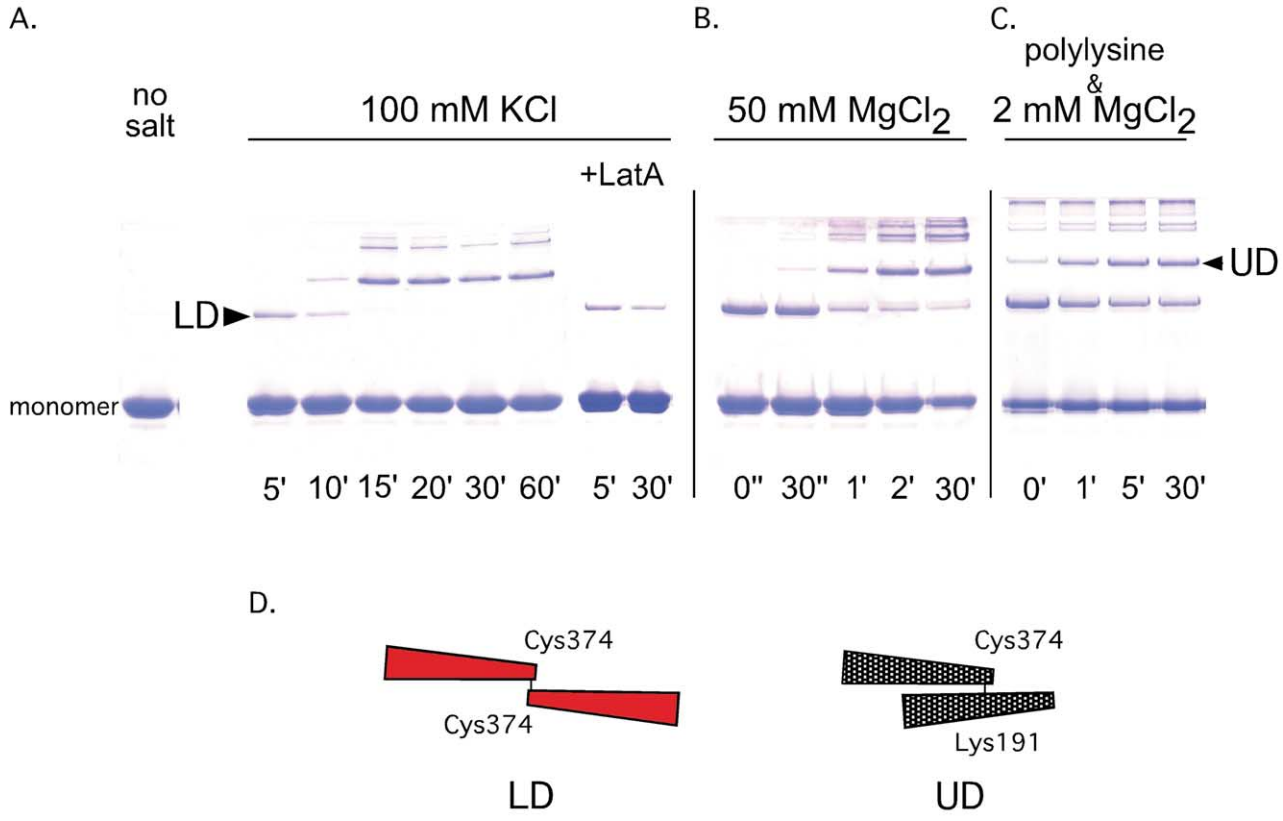

Fig. 1. Actin polymerization monitored by 1,4-PBM cross-linking. Actin (1 mg/ml) was polymerized under different conditions. Aliquots were removed at the time points indicated and cross-linked with 1,4-PBM as previously described [9]. Covalently cross-linked products were analyzed on SDS–PAGE gels. A: The polymerization reaction was induced by adding KCl to Ca-G-actin to a final concentration of 100 mM. Under these conditions, the band representing the first intermolecular cross-linked species migrates with an apparent molecular mass of ~86 kDa. This so-called 'lower dimer' (LD) is detected immediately after salt addition (nominal time point zero; not shown) and persists during the first 5 min of the polymerization reaction. Then a second band with a slightly slower electrophoretic mobility (~120 kDa) appears. At later time points, this 'upper dimer' (UD) is the only dimer present and in addition higher oligomers are cross-linked. The lane on the far left represents monomeric Ca-G-actin incubated with 1,4-PBM in the absence of KCl. Right panel, in the presence of LatA only LD forms at 100 mM KCl and persists over time. B: Polymerization was initiated by adding 50 mM $MgCl_2$ to Ca-G-actin. LD assembly during the nucleation phase is more efficient (relative to monomer) under these conditions and the shift from LD to UD occurs much faster. Note that under these polymerization conditions, which yield paracrystalline filament arrays, some LD persists at steady state. C: Mg-G-actin (Mg^{2+}) occupying the high affinity binding site was polymerized by the addition of 12 μM polylysine and 2 mM $MgCl_2$. Under these conditions LD formation is instant and persists so that at steady state UD and LD coexist in comparable amounts. D: Schematic model of cross-linked dimers based on cross-linking experiments and structural considerations (see [10]). The subunits of the LD are cross-linked via the Cys374 residues in an antiparallel configuration, whereas the subunits of the UD are parallel, with Cys374 of one subunit being cross-linked to Lys191 of the other subunit.

latrunculin A (LatA), a monomer sequestering drug isolated from a red sea sponge [15], arrests polylysine-induced polymerization at the stage of LD production [6]. Likewise, in the presence of LatA the shift to UD is abolished when Ca-G-actin is polymerized with 100 mM KCl (Fig. 1A). Together, these findings indicate that, while LatA prevents those actin–actin contacts that result in cross-linking of UD, it does not impede actin–actin interactions that yield LD. Evidently, LD and UD represent two distinct dimers with their actin subunits being in different conformations and different configurations relative to one another.

Comparison of the time course of dimer formation with other polymerization assays (such as the fluorescence enhancement upon incorporation of pyrenated actin subunits into filaments) reveals that the time of the shift from LD to UD correlates well with the transition from the nucleation to the elongation phase [9,14]. Electron microscopy studies confirmed that the appearance of UD in cross-linking experiments is accompanied by the formation of relatively long filaments.

In summary, cross-linking experiments have revealed that the first oligomer detected by 1,4-PBM is a transient dimer, the LD, which arises during the nucleation phase but gradually disappears during the elongation phase, except under polymerization conditions that yield paracrystalline actin filament arrays. Subsequently, a different actin dimer, the UD, is produced with much slower kinetics. In contrast to UD, which readily polymerizes into filaments, LD by itself is unproductive.

2. Biochemical properties of actin dimers

Cross-linking with 1,4-PBM was first used in the 80's to gain information on the molecular architecture of the actin filament. Accordingly, cross-linking prepolymerized actin filaments and then depolymerizing them predominantly yielded a dimer, which was later identified to correspond to the UD detected during polymerization [16]. When 1,4-PBM cross-linked UD was added to a monomer solution it increased nucleation and filament polymerization [17]. Even on its

own, purified cross-linked UD assembled into filaments that are comparable to synthetic F-actin filaments polymerized from G-actin, confirming that this dimer nucleates polymerization and corresponds to a true building block of the F-actin filament [9]. Taken together, these data indicated that the contacts between the two actin subunits within the UD are similar to those between two neighboring subunits along the short-pitch genetic helix of the F-actin filament. Subsequently, the residues cross-linked by 1,4-PBM were mapped to be Lys191 of one subunit and Cys374 of the other (see Fig. 1D; [12]). Recently, the length of the 1,4-PBM linker has been re-evaluated and found to be 11.1 ± 0.5 Å [18]. With this figure and the atomic model of the actin filament [19,20], one could now test the conclusions drawn from earlier studies. However, the flexibility of the C-terminus where the Cys374 residue resides and the resulting structural ambiguities might render this a difficult confirmation.

The different biochemical behavior of LD compared to UD and the evidence that Cys374 is the only cysteine residue in actin involved in the cross-linking reaction led to the conclusion that the two actin subunits in LD are cross-linked via Cys374 in an antiparallel configuration (see Fig. 1D; [21]). Recently, the crystal structure of an actin dimer formed in the presence of LatA and polylysine, which has biochemical features similar to those of the LD, was solved at 3.5 Å resolution [6]. The crystallographic structure revealed an antiparallel, end-to-end actin dimer stabilized by a disulfide bond between the two Cys374 residues. In contrast, besides being unable to form a disulfide bond because of their interaction with the 1,4-PBM cross-linker, the two Cys374 residues in LD are spaced by ~ 11.1 Å, suggesting that the crystallographic dimer identified by Bubb and colleagues [6] is not identical to that occurring in solution. Whether this difference can be explained by the flexibility of the C-terminus of actin or whether it reflects two distinct antiparallel dimers, which are perhaps interconvertible, remains an open question.

A dimer with an electrophoretic mobility similar to that of LD was also obtained by reversibly cross-linking two actins via their Cys374 by a bifunctional thiol-specific reagent [22]. Like purified 1,4-PBM cross-linked LD, this isolated dimer was unable to polymerize. However, in this case the cross-linking was reversible and the actin subunits, having returned to their native conformation, were able to polymerize into F-actin filaments.

It has recently been reported that slow oxidation of G-actin yields dimers with filament cross-linking activities [23]. Intriguingly, the main species detected by non-reducing SDS–PAGE was a disulfide-bonded dimer with an electrophoretic mobility similar to that of LD. Filaments polymerized from G-actin that was not protected from oxidation were reported to form 'end-to-side filament connections' reminiscent of filament branching. Interestingly, this study suggests that the polymerization kinetics are unaffected by the presence of the disulfide-bonded actin dimer species.

Dimeric actin species have also been found in association with actin-binding proteins (ABPs). For example, gelsolin forms a ternary complex with two actin monomers in the presence of calcium, which nucleates actin polymerization at the pointed end and caps the barbed end of filaments [24]. Addition of 1,4-PBM to this ternary complex yielded a single cross-linked dimeric actin species with an electrophoretic mobility indistinguishable from that of LD [21]. Consistently, cross-linked LD bound to gelsolin in a 1:1 stoichiometry, but this complex did not nucleate filament assembly. Contrary to what had been assumed until then, these studies revealed that the two actin subunits complexed with gelsolin are in an antiparallel orientation. What this finding also suggests is that ABPs might play a role in stabilizing the otherwise transient LD conformation.

At this stage, biochemical analysis leaves us with the knowledge that there is an antiparallel actin dimer produced during the nucleation phase of filament polymerization. Its particular conformation opens the door for structural polymorphism. However, if stabilizing downstream factors are missing, for example in polymerization reactions with purified actin, the LD goes unnoticed.

3. Morphological consequences of LD on actin assembly

The detection of a transient LD at early stages of polymerization in vitro imperatively led to the question of its significance in actin assembly, mainly since purified cross-linked LD did not form conventional filaments under polymerizing conditions. Moreover, cross-linking of prepolymerized F-actin filaments with 1,4-PBM and subsequent depolymerization predominantly yielded UD [11,16], suggesting that the LD conformation does not significantly occur in mature F-actin filaments at steady state. Evidently, the actin–actin interactions in the LD are distinct from those along and between the two long-pitch helical strands of the filament.

However, given the appropriate conditions, purified LD does assemble into a range of polymorphic supramolecular structures (Fig. 2; [10]), which can be taken as a first indication of the LD's involvement in supramolecular actin patterning. For example, polymerization conditions that favor the lateral association of F-actin filaments into paracrystalline arrays (i.e. 50 mM $MgCl_2$) also induce the formation of laterally aligned structures from purified cross-linked LD, albeit less ordered. Intriguingly, 1,4-PBM cross-linking under these conditions revealed a higher yield of LD compared to 100 mM KCl and also a significant amount of LD persisted at steady state (compare Fig. 1A,B; [10]). Lateral aggregation of filaments was also observed when filaments were polymerized in the presence of polylysine [13,25]. As noted for 50 mM $MgCl_2$, polylysine also induced significant amounts of LD throughout the polymerization reaction with even a higher yield of LD at steady state (Fig. 1C). The reason for this increased yield of LD over UD at steady state may have to do with the fact that polylysine-induced paracrystalline arrays are much more extensive than those induced with ≥ 10 mM $MgCl_2$. In combination with LatA, polylysine-induced polymerization led to an accumulation of LD [6] and at the same time, filaments and paracrystals were no longer formed (our unpublished observations). Based on the evidence described above it seems conceivable that the LD conformation is involved in interactions between filaments, lending further support to its role in supramolecular patterning of actin filaments.

'Unconventional' intersubunit contacts, as opposed to the conventional ones along and between the two long-pitch helical strands of filaments, are also formed in so-called tubes and sheets, crystalline actin assemblies induced by the trivalent lanthanide gadolinium [26,27]. Cross-linking of sheets yielded significant amounts of LD [10]. Moreover, cross-linked LD assembled into ribbon-like structures similar to

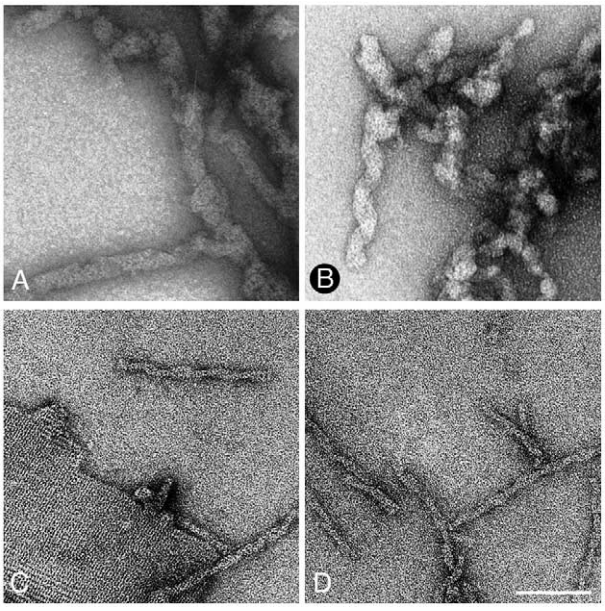

Fig. 2. LD-based structural polymorphism. A: Folded ribbons obtained with cross-linked LD in the presence of gadolinium under actin sheet preparation conditions. B: Folded ribbons induced by gadolinium using normal monomeric Ca-G-actin. They are likely to represent a precursor form of crystalline actin sheets. C: Actin sheets prepared from Ca-G-actin in the presence of LatA. Note the presence of distinct filamentous polymers exhibiting 'paired helical filament-like' appearance with a characteristic twist and axial repeat. D: Gadolinium-induced actin sheets were treated with LatA. The resulting LD-related structures are comparable to those in (C). Scale bar, 100 nm.

those observed at early stages of sheet formation (Fig. 2A,B). The notion that LD-like contacts are present in crystalline sheets and tubes gained further support by recent studies in which we built an atomic model of crystalline actin tubes by fitting the atomic structure of the monomer into an EM-based three-dimensional reconstruction obtained from tilt series of negatively stained tubes [7,8]. These studies indicated that the arrangement of actin monomers within the tubes involves antiparallel packing into dimers with *p2* symmetry. Based on this model, most of the intersubunit contacts within or between these dimers are absent in the actin filament and therefore might correspond to the contacts in the LD. Preliminary data from our laboratory suggest that upon incubation of crystalline actin sheet preparations with LatA, the packing of sheets and ribbons is disrupted and relatively short, filamentous structures with a helical twist and characteristic periodicity arise (Fig. 2C,D). The dimensions and geometry of these new structures are clearly distinct from those of the F-actin filament. From our knowledge about the effects of LatA (see above) we might speculate that it only interferes with the intersubunit contacts within the sheets that correspond to the long-pitch helix intersubunit contacts in the filament but not with the LD-like contacts.

An important cue to a possible role of LD during actin polymerization was provided by electron microscopy studies (Fig. 3; [9]). Negatively stained specimens prepared at different time points after initiation of polymerization of Ca-G-actin with 100 mM KCl revealed that during early stages, i.e. at time points when predominantly LD formation is occurring (see Fig. 1A), the growing filaments exhibit a ragged appearance (Fig. 3, 5 min). The 'smooth', regular morphology we all associate with pure F-actin filaments is only observed at later time points (Fig. 3, 60 min), when in cross-linking experiments LD is no longer detected but has been replaced by UD and presumably higher oligomers (Fig. 1A; [9]). These time-resolved electron micrographs suggest that the LD is in fact transiently incorporated into growing filaments, presumably via one of its subunits, with the second subunit 'jutting out', which gives the filament a 'ragged', 'decorated' appearance. Further evidence that the ragged morphology observed in growing filaments is correlated with the incorporation of LD is provided by electron micrographs of actin filaments at steady state, where the LD conformation has been stabilized by the addition of 1,4-PBM and phalloidin at the onset of polymerization. Under these conditions, even mature filaments exhibit a ragged morphology. These experiments not only indicate that the LD is incorporated into the growing filament via one of its actin subunits, but they also suggest that this LD configuration can 'nucleate' daughter filaments that branch off the mother filament. Because there were no other proteins, specifically no ABPs, present in the polymerization reaction, these observations suggested to us that it is actually the actin itself, via the LD conformation, which initiates its supramolecular patterning.

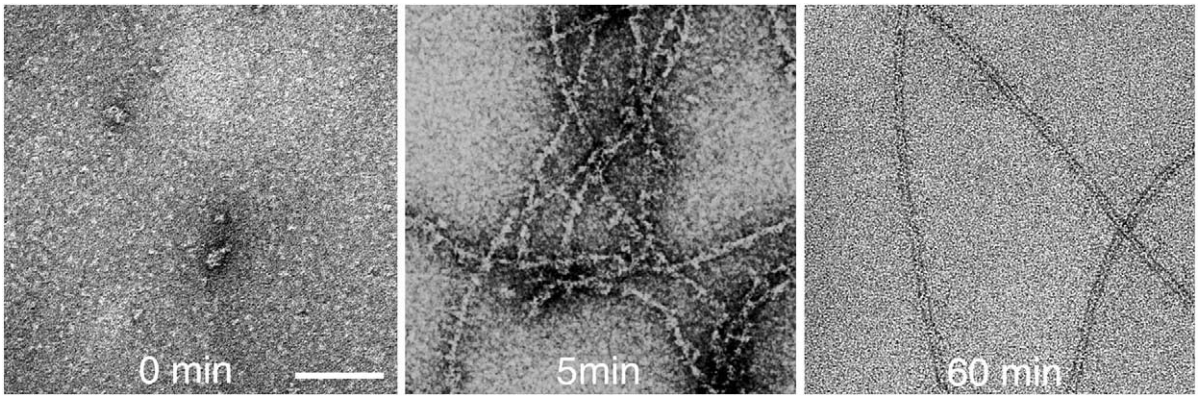

Fig. 3. Time course of polymerizing F-actin filaments. Negatively stained specimens of polymerizing actin filaments were imaged by transmission electron microscopy at different time points. (left) Immediately after addition of 100 mM KCl to Ca-G-actin. (middle) 5 min after the onset of polymerization, the growing filaments exhibit a ragged morphology with indications of branching sites along the filaments. (right) At 60 min (steady state), dispersed F-actin filaments without branches and a smooth morphology predominate. Scale bar, 100 nm.

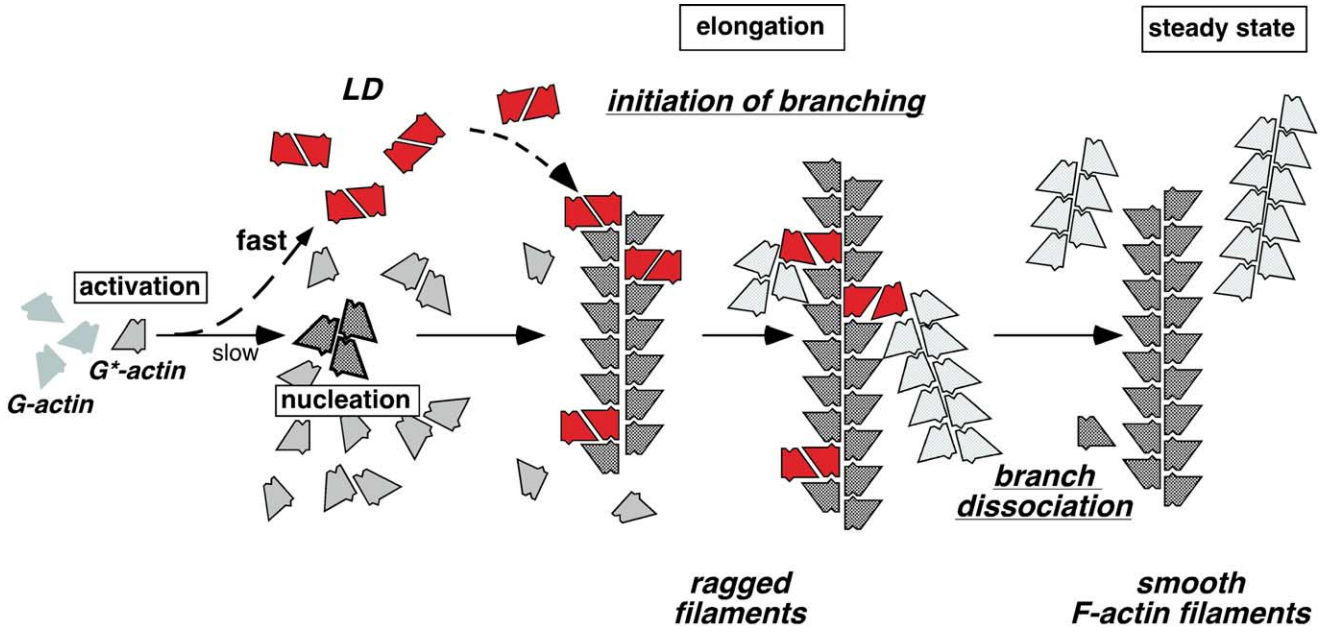

Fig. 4. A dual pathway for actin assembly. Following the conventional polymerization pathway, activated G*-actin slowly nucleates before elongation occurs by the addition of monomers and small oligomers such as the UD. At steady state, F-actin filaments are disperse, unbranched and exhibit a smooth morphology. On a sidetrack, actin rapidly forms an antiparallel LD which, still in a G-like conformation, incorporates into the growing filaments via one of its subunits. During their transient stay in the filament, the antiparallel dimers initiate the branching of daughter filaments, thereby causing the ragged appearance of the mother filament. After a conformational change (most probably from a G-like to an F-like conformation), the LD dissociates and releases the daughter filament or the 'free' subunit in those cases where LD has not nucleated a new filament. At steady state, only dispersed, unbranched F-actin filaments with a smooth morphology are observed.

4. The LD pathway: key to actin filament patterning

In the general view, the basis of actin's fundamental role in biology lies in its inherent ability to rapidly assemble and disassemble linear filaments. However, correlating the cross-linking data described above with morphology provides compelling evidence that actin can do more than just polymerize into filaments. For instance, in vitro it first assembles LD during the nucleation phase, independent of the type of actin used [14], the divalent cation (Mg^{2+} or Ca^{2+}) occupying the high affinity binding site, and the polymerization conditions chosen (albeit the kinetics and yield of LD formation are modulated by these parameters). Most notably, the LD is in a G-actin-like rather than an F-actin-like configuration [10]. Because purified LD was shown to assemble laterally aligned structures but not F-actin filaments and LD-like contacts are present in crystalline actin sheets, one could envisage that LD is predominantly involved in interactions between filaments rather than within filaments.

The generally slower appearance of UD relative to LD, and in contrast to LD a true filament precursor, tells us that actin's inherent properties allow it to follow at least two different pathways, which are illustrated in Fig. 4. The dual pathway model outlines how, by following the so-far undervalued 'LD pathway', actin could initiate its own filament patterning.

Why then has it, particularly in vivo, gone unnoticed for the most part? Following the UD pathway, activated G-actin assembles a trimeric nucleus (Fig. 4), in which all the actin–actin contacts occurring within the F-actin filament have already been established. In vivo this otherwise slow reaction is accelerated by a number of ABPs. Subsequently, monomers or small filament precursors, such as the UD, are added on, until a steady state is reached where filaments stop growing. Electron micrographs reveal disperse, linear filaments at steady state, which exhibit the well-known regular morphology (Fig. 3C; [9,28]). Where then, if at all, does the LD pathway join the UD during filament formation? Starting out on its own pathway, LD evidently incorporates into the growing filament via one of its subunits with its second subunit jutting out. This (or any immediately following) configuration either dissociates and, by doing so, the subunit residing in the filament continues along the UD pathway, or the incorporated LD nucleates a daughter filament emanating from the mother filament. Accordingly, filaments at this stage exhibit a ragged morphology and occasional branching sites can be detected (Fig. 3, 5 min). Upon conformational changes of the LD, the interfilament contact falls apart and at steady state bona fide unbranched F-actin filaments are observed (Fig. 3, 60 min).

Moreover, interfilament contacts are established in laterally aligned filament bundles. Several pieces of evidence point at an involvement of LD in assembling such filament arrays. For example, cross-linking of paracrystalline actin filament arrays yields a significant amount of LD [10]. When polymerization is induced under conditions that favor the lateral association of filaments into paracrystalline arrays (≥ 10 mM $MgCl_2$ or the presence of polylysine [13,25,29]), a significant amount of LD is observed at steady state by 1,4-PBM cross-linking (Fig. 1B,C). Interestingly, Bubb and colleagues showed that the presence of LatA appeared to arrest polylysine-induced polymerization at the LD stage [6]. Moreover, the antiparallel configuration of the actin subunits in the LD is consistent with the lateral interaction of two neighboring filaments with opposite polarity. In this case LD acts as a cross-linker, with one of its subunits residing in one filament and the second subunit being part of the neighboring antiparallel filament.

Our model proposes that actin on the LD pathway is its own initiator of supramolecular filament patterning. By acting as an initiator of branching and as an actin 'bundling factor' via sharing a monomer each with two adjacent filaments, LD sets off a variety of supramolecular assemblies. The final outcome, however, depends on the actin binding proteins standing in alert.

As might have been noted, the LD configuration assumed in the model in Fig. 4 leads to an antiparallel filament branching which is in conflict with the parallel filament branching observed in dendritic actin filament networks involving the Arp2/3 complex [3,30,31]. In our scheme, however, we have employed an antiparallel LD configuration so that its two-fold axis of symmetry is oriented perpendicular to the F-actin filament axis. While this LD configuration is formally consistent with that exhibited by the recently reported crystal structure of an LD [6], it is conceivable that a second LD configuration may exist with the corresponding two-fold axis of symmetry being oriented parallel to the F-actin filament axis. Such an LD configuration, for which we have some preliminary evidence [8], would indeed produce parallel filament branching.

5. Actin filament patterning in cells

Among the supramolecular actin assemblies that have received most attention in recent years are the intricate meshwork of extensively branched actin filaments at the leading edge of motile cells [30] and the actin comet tails of the intracellular pathogen *Listeria monocytogenes* [32]. A number of excellent reviews summarize the fundamental insights on actin-based motility gained from these two systems [33–37]. In both cases, the dynamic reorganization of the actin filament meshwork, in particular the generation of free barbed ends that allow rapid filament elongation near the plasma membrane or at one pole of the pathogen, provides the basis for actin polymerization-driven motility. Several mechanisms act in concert to guarantee rapid actin assembly at the leading edge and with it, the propulsive force that pushes forward the lamella of migrating cells. One of these mechanisms involves the de novo nucleation of branching filaments. Considering the dendritic actin meshwork, this mechanism frequently comes into action at the leading edge in moving cells.

Over the past years, since its isolation in 1994 [1], the heptameric Arp2/3 complex has emerged as a key organizer of the branched actin filament meshwork [2–5,33–37]. First it was shown in vitro with purified components that the complex binds to the side as well as to the pointed end of actin filaments and that it attaches the pointed end of one filament at a 70° angle to the side of another [38]. The complex was also found in association with branch sites near the plasma membrane of moving cells [30]. Recent studies directly visualized filament branching mediated by the Arp2/3 complex [31,39–41]. Despite the convincing evidence that Arp2/3 is somehow involved in the establishment of branches and despite the structural data available on the complex [42,43], the molecular mechanism of filament nucleation remains an enigma. One current idea is that the actin-related subunits Arp2 and Arp3 form a heterodimer that mimics a conventional dimer [44]. While this is an attractive concept, we like to think that the Arp2/3 complex acts to stabilize the arising structure rather than initiating it. More likely, the true initiator of branching is actin itself, when it is following the LD pathway. In support of this concept, actin is perfectly able to form branches in the absence of Arp2/3 complex or any other ABP (Fig. 5). Moreover, since other ABPs, for example filamin [45], also promote branching of actin filaments, one could imagine that a common principle for the initiation (the LD) of branching lies with the actin (i.e. via the LD incorporation into growing – or dynamic – actin filaments), and that the different ABPs are responsible for the temporal and spatial regulation of distinct actin filament patterning.

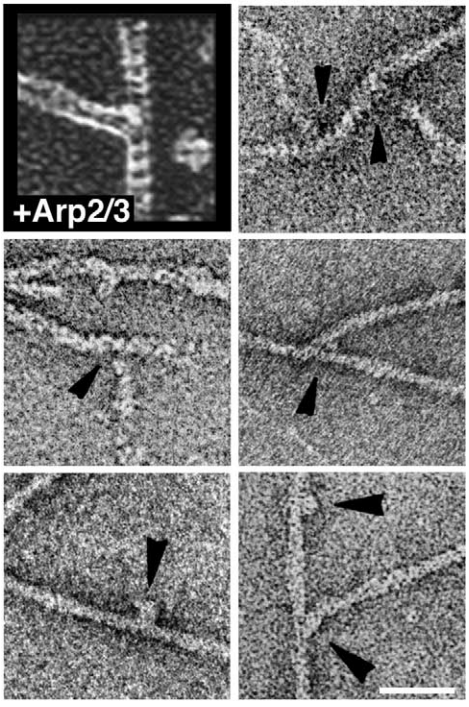

Fig. 5. Gallery of F-actin filament branches. Polymerization of purified actin was induced by 100 mM KCl. After 3 min, polymerizing actin was cross-linked by 1,4-PBM for 2 min before a stoichiometric amount of phalloidin was added, so that LD remained incorporated in the F-actin filaments at steady state (for experimental details see [9]). Electron micrographs of negatively stained samples reveal branching of filaments. Scale bar, 30 nm. For comparison, an electron micrograph of quick-frozen, deep-etched, and rotary-shadowed filament branching in the presence of the Arp2/3 complex is shown in the top left panel (adapted from [38]).

6. Unconvential actin assemblages in the nucleus

Both fluorescently labeled phalloidin as well as electron microscopy have failed to visualize conventional actin filaments in the nucleus. The absence of detectable filaments has fueled decades of controversy regarding the plain existence of nuclear actin which many researchers considered to represent a contamination due to the high concentration of actin in the cytoplasm. The recent detection of actin in a number of eukaryotic chromatin remodeling and modifying complexes (reviewed in [46]) has finally settled the debate over actin being or not being in the nucleus in favor of its existence and has instigated possible functions of nuclear actin. Because the interphase chromosomes create a curvilinear, sinusoidal interchromatin space [47], F-actin, with a persistence length of typically 3–5 μm [9], would not traject very far without hitting a wall of chromatin, and the same would probably hold true

for dendritically branched actin. Since many ABPs that are implicated in the assembly of filamentous actin structures in the cytoplasm are also found in the nucleus, the apparent lack of filaments is even more puzzling. If oligomeric or polymeric actin is indeed present in the nucleus (unknown at present), it seems likely that its conformation is distinct from that of classical F-actin.

This conclusion gained support by the recent work of Gonsior et al. [48]. Using a reconstituted profilin:actin complex they raised a monoclonal antibody against actin (2G2) which recognizes a non-sequential actin epitope. In the atomic model of the F-actin filament [19,20] this epitope is not presented as a cohesive structure. In fibroblasts and myogenic cells, 2G2 did not react with actin stress fibers or myofibrils, respectively, whereas it stained distinct, dot-like structures in the nucleus. A staining associated with the nucleoplasmic side of nuclear envelopes from *Xenopus* and *Pleurodeles* oocytes was also observed by immunogold electron microscopy [49]. Furthermore, gold-conjugated 2G2 did not bind to synthetic F-actin filaments (our unpublished results). Together, these data suggest that this antibody recognizes native actin in a conformation that is distinct from the F-actin conformation. One such actin conformation is that of the LD since its actin subunits are in a G-like conformation and involve actin–actin interactions that do not occur within mature F-actin filaments. In addition, LD only transiently appears in growing filaments. Granting that 2G2 recognizes native LD, one is tempted to speculate that, with its propensity to induce supramolecular filament patterning, LD might indeed be a key conformation in the structural organization of nuclear actin. Current efforts in our lab are directed towards testing this idea. Maybe 2G2, or any other LD-specific antibody, will prove a valuable tool to investigate the structure and organization of nuclear actin, as well as to analyze the functional significance of LD in the nucleus.

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